REMARKS

The Office Action

Claims 1-20 are pending. Claims 1-20 stand rejected for indefiniteness. Claims 1, 2, 7, 9, and 14-20 also stand rejected for anticipation by Sorenson et al. (U.S. Patent No. 5,496,699; hereafter "Sorenson"). Claims 10-13 stand further rejected for obviousness over Sorenson in view of Mullis et al. (U.S. Patent No. 4,965,188; hereafter "Mullis"). Claims 3-6 would be allowable if rewritten in independent form and to overcome the rejection for indefiniteness. Claim 11 is objected to as being of improper dependent form.

Abstract

The application has been objected to under 37 C.F.R. 1.72(b) as lacking an abstract at the end of the specification. This error is regretted and has been corrected by the present amendment.

Claim Objections

Claim 11 is objected to as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicants cancel claim 11, and this objection is now moot.

Rejections under 35 U.S.C. §112, second paragraph

Claims 1-20 are rejected for indefiniteness. Claims 1-20 are rejected for the recitation of the language "opposite strands" and "bordering the position of said polymorphic sequence" in claims 1 and 14. The language "opposite strands" and "bordering the position of said polymorphic sequence" was used to describe the sites where primers bind to a nucleic acid sequence. This language has been replaced by the phrase "a first member of said first pair of PCR primers hybridizing to one strand of said nucleic acid sequence and the other member of said first pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said first pair hybridizes." This phrase indicates that, for a double-stranded sequence of DNA, one of the pair of primers hybridizes to each strand, such that the extension of each primer yields a sequence of DNA to which the other primer can bind.

Claims 1-20 are also rejected for indefiniteness for use of "being non-complementary to said nucleic acid sequence at a single non-complementary nucleotide in its 3'-terminal nucleotides 2-6" in claims 1, 6, 8, 10, 14, and 16-18. This phrase has now been replaced by "[is] non-complementary to said nucleic acid sequence at a single nucleotide that is disposed within the five nucleotides adjacent to said 3'-terminal nucleotide." This replacement phrase clearly indicates the location of the non-complementary nucleotides.

Claims 2-5 are further rejected for indefiniteness, with the Examiner stating, "[I]t is unclear what is meant by the language 'different ranges of specificity' in claim 2."

This rejection is respectfully traversed. The definition of "range of specificity" is given on page 6 of the specification. This definition reads:

> By "range of specificity" is meant the range of nucleic acid template:PCR primer ratios at which template sequences differing by at least one nucleotide may be discriminated by assaying for the presence of detectable PCR amplification product formation.

This definition is illustrated for one set of primers in Figure 3, where the specificity ranges from ~ 0.5 - ~ 400 ng of DNA. Over this range, the amount of amplified target DNA is above a set detection level, while the amount of amplified non-target DNA is below the detection level. Figure 4 is an illustration of two sets of primers having different ranges of specificity. One set has a range of specificity of 0.001 – 1 ng DNA, and the other has a range of specificity of 0.01 – 10 ng DNA. The Examiner also asserts that the recitation of "3000-fold range of specificity" in claim 4 is unclear. Figure 4 illustrates the meaning of a greater than 3000-fold range of specificity. In this figure, the combined range of specificity for the two sets of primers is 0.001 – 10 ng DNA, which is a 10,000-fold range.

Claims 8 and 9 are also rejected for indefiniteness because of the language "a universal primer binding site." Applicants assert that "universal primer" is a term used in the art for a single set of primers that can be used to amplify many different nucleic acid sequences. Thus, a universal primer binding site is a sequence of nucleotides that is complementary to a universal primer. Applicants submit herewith a passage from Watson et al. (Recombinant DNA, 2nd ed., W.H. Freeman:New York 1992) and an abstract from Francis et al. (J. Ind. Microbiol. Biotechnol. 1997, 19:286-293) as examples

of how the term "universal primer" is routinely used in the art. Applicants use of this term in the present claims is consistent with its art-recognized usage.

Claims 10-13 are further rejected for indefiniteness on the basis that "it is unclear what is meant by the language 'a unique hybridization tag'. Does it mean that the hybridization tag has a label on it?" In response, Applicants direct the Examiner's attention to the definition of hybridization tag, given on page 6 of the specification. There the specification states:

> By "hybridization tag" is meant an oligonucleotide that differs sufficiently in sequence from a target nucleic acid (for example, a target nucleic acid to be amplified) that significant cross-hybridization does not occur. When multiple hybridization tags are utilized in a single reaction mixture, these tags also preferably differ in sequence from one another such that each has a unique binding partner. (Emphasis added)

A hybridization tag thus is an oligonucleotide whose uniqueness stems from its nucleic acid sequence.

In view of the foregoing remarks, the rejection of claims 1-20 for indefiniteness should be withdrawn.

Rejections under 35 U.S.C. § 102(b)

Claims 1-2, 7, 9, and 14-20 stand rejected for anticipation by Sorenson. Applicants traverse this rejection. "A claim is anticipated only if each and every claim element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987) Sorenson does not meet this standard.

Claims 1 and 15, the independent claims rejected, are directed to a method and a kit for determining whether a nucleic acid sequence comprises a particular allele of a polymorphic sequence. Each of these claims requires first and second pairs of primers, characterized as follows:

> (i) one of said first pair of PCR primers is (a) complementary at its 3'-terminal nucleotide to a first allele of said polymorphic sequence, (b) non-complementary at its 3'-terminal nucleotide to a second allele of said polymorphic sequence, and (c) non-complementary to said nucleic acid sequence at a single nucleotide that is disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said first pair of primers is capable of amplifying said first allele under appropriate conditions; and (ii) one of said second pair of PCR primers is (a) complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, (b) non-complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, and (c) non-complementary to said nucleic acid sequence at one or more nucleotides that are disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said second pair of primers is capable of amplifying said first allele under appropriate conditions

Thus, claims 1 and 15 require two primers that have the same nucleotide at their 3' termini but differ in the number of non-complementary nucleotides located within the five nucleotides adjacent to the 3'-terminal nucleotide.

The Examiner states, "The method [of Sorenson] applies four primer pairs including a set of four allele-specific first primers complementary to the gene sequence contiguous with the site of the mutation on the first strand." Sorenson teaches that "[t]hese four primers are unique with respect to each other and differ ... at the 3' nucleotide which is complementary to the wild type nucleotide or to one of the three possible mutations which can occur at this known position." (Col. 2, ll. 30-34; Emphasis added) Since the four primers of Sorenson are unique at their 3' nucleotides, no two

primers of Sorenson have the same 3'-terminal nucleotide, as required by claims 1 and 15. Thus, Sorenson does not disclose all of the limitations of claims 1 and 15, and the rejection for anticipation should be withdrawn.

Rejections under 35 U.S.C. §103(a)

Claims 10-13 stand rejected as being obvious over Sorenson in view of Mullis. Applicants respectfully disagree. Claim 10, from which claims 11-13 depend, is directed to the method of claim 1 with the additional limitation that certain of the primers include a unique hybridization tag.

In rejecting claims 10-13, the Examiner states that the claimed methods are *prima facie* obvious in view of the combination of the methods and primers taught by Sorenson and the detection of amplified product by using sequence specific oligonucleotides affixed to a membrane as taught by Mullis. As stated above, Sorenson does not disclose the methods of the present invention, since it does not teach or suggest the use of two different primers that are complementary to one allele at their 3'-terminal nucleotides. The teachings of Mullis on oligonucleotides affixed to membranes (or any other teachings of Mullis) do not remedy the deficiencies of Sorenson, and thus, the claimed methods are patentable over the cited art. The §103 rejection should be withdrawn.

CONCLUSIONS

Applicants submit that the claims are in condition for allowance and such action is requested. If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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Version with markings to show changes

Marked-up versions of claims 1, 6-8, 10, 12, and 14-18 are as follows.

1. (Amended) A method for determining whether a nucleic acid sequence comprises a particular allele of a polymorphic sequence, said method comprising:

(a) contacting said [a] nucleic acid sequence, in one amplification reaction [the same] or [a] separate amplification reactions, with a first pair of PCR primers and a second pair of PCR primers under conditions that allow hybridization of said PCR primers to said nucleic acid sequence, a first member of said first pair of PCR primers hybridizing to [opposite strands] one strand of said nucleic acid sequence and the other member of said first pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said first pair hybridizes [bordering the position of said polymorphic sequence], and a first member of said second pair of PCR primers hybridizing to [opposite strands] one strand of said nucleic acid sequence and the other member of said second pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said second pair hybridizes [bordering the position of said polymorphic sequence], said PCR primers being characterized as follows:

(i) one of said first pair of PCR primers is (a) [being] complementary at its 3'-terminal nucleotide to a first allele of said polymorphic sequence, (b) [being] non-complementary at its 3'-terminal nucleotide to a second allele of said polymorphic sequence, and (c) [being] non-complementary to said nucleic acid sequence at a single [non-complementary] nucleotide that is disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said first pair of primers is capable of amplifying said first allele under appropriate conditions [in its 3'-terminal nucleotides 2-6]; and

(ii) one of said second pair of PCR primers is (a) [being] complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, (b) [being] non-complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, and (c) [being] non-complementary to said nucleic acid sequence at one or more nucleotides that are disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said second pair of primers is capable of amplifying said first allele under appropriate conditions [in its 3'-terminal nucleotides 2-6];

(b) carrying out said amplification reaction or reactions; and

(c) detecting any [an] amplification product of step (b), wherein [as an indication of] the presence of amplification product is indicative of the presence [, in said nucleic acid sequence,] of said first allele in said nucleic acid sequence [of said polymorphic sequence].

6. (Amended) The method of claim 1, wherein said one of said second pair of PCR primers includes at least two non-complementary nucleotides that are disposed

within the five nucleotides adjacent to the 3'-terminal nucleotide of each primer [in its 3'-terminal nucleotides 2-6].

7. (Amended) The method of claim 1, wherein said polymorphic sequence comprises [method is carried out to identify] a single nucleotide polymorphism.

8. (Amended) The method of claim 1, wherein said one of said first pair of PCR primers in step (a)(i) and said one of said second pair of PCR primers in step (a)(ii) [each of said primers of said first and said second primer pairs that comprises a non-complementary nucleotide in its 3'-terminal nucleotides 2-6] also comprise[s] a universal primer binding site.

10. (Amended) The method of claim 1, wherein said one of said first pair of PCR primers in step (a)(i) and said one of said second pair of PCR primers in step (a)(ii) [each of said primers of said first and said second primer pairs that comprises a non-complementary nucleotide in its 3'-terminal nucleotides 2-6] also comprise[s] a unique hybridization tag.

12. (Amended) The method of claim 10 [11], wherein said detection step is carried out on a solid support to which a binding partner for each hybridization tag is immobilized.

14. (Amended) The method of claim 1, further comprising:

(d[a]) contacting said nucleic acid sequence, in one amplification reaction [the same] or [a] separate amplification reactions, with a third pair of PCR primers and a fourth pair of PCR primers under conditions that allow hybridization of said PCR primers to said nucleic acid sequence, a first member of said third pair of PCR primers hybridizing to [opposite strands] one strand of said nucleic acid sequence and the other member of said third pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said third pair hybridizes [bordering the position of said polymorphic sequence], and a first member of said fourth pair of PCR primers hybridizing to [opposite strands] one strand of said nucleic acid sequence and the other member of said fourth pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said fourth pair hybridizes [bordering the position of said polymorphic sequence], said PCR primers being characterized as follows:

(i) one of said third pair of PCR primers is (a) [being] complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) [being] non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) [being] non-complementary to said nucleic acid sequence at a single nucleotide that is disposed within the five nucleotides adjacent to said 3'-terminal

nucleotide, wherein said third pair of primers is capable of amplifying said second allele under appropriate conditions [in its 3'-terminal nucleotides 2-6]; and

(ii) one of said fourth pair of PCR primers is (a) [being] complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) [being] non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) [being] non-complementary to said nucleic acid sequence at one or more nucleotides that are disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said fourth pair of primers is capable of amplifying said second allele under appropriate conditions [in its 3'-terminal nucleotides 2-6]; and

(e[b]) carrying out said amplification reaction or reactions; and

(f[c]) detecting any [an] amplification product of step (e), wherein [as an indication of] the presence of amplification product is indicative of the presence [, in said nucleic acid sequence,] of said second allele in said nucleic acid sequence [of said polymorphic sequence].

15. (Amended) A kit for determining whether a nucleic acid sequence comprises a particular allele of a polymorphic sequence, said kit comprising:

(a) a first pair of PCR primers and a second pair of PCR primers, a first member of said first pair of PCR primers hybridizing to [opposite strands] one strand of said nucleic acid sequence and the other member of said first pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said first pair hybridizes [bordering the position of said polymorphic sequence], and a first member of said second pair of PCR primers hybridizing to [opposite strands] one strand of said nucleic acid sequence and the other member of said second pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said second pair hybridizes [bordering the position of said polymorphic sequence], wherein said PCR primers are [being] characterized as follows:

(i) one of said first pair of PCR primers is (a) [being] complementary at its 3'-terminal nucleotide to a first allele of said polymorphic sequence, (b) [being] non-complementary at its 3'-terminal nucleotide to a second allele of said polymorphic sequence, and (c) [being] non-complementary to said nucleic acid sequence at a single [non-complementary] nucleotide that is disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said first pair of primers is capable of amplifying said first allele under appropriate conditions [in its 3'-terminal nucleotides 2-6]; and

(ii) one of said second pair of PCR primers is (a) [being] complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequenc, (b) [being] non-complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, and (c) [being] non-complementary to said nucleic acid sequence at one or more nucleotides that are disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said second pair of primers is capable of amplifying said first allele under appropriate conditions [in its 3'-terminal nucleotides].

16. (Amended) The kit of claim 15, further comprising:

(b[a]) a third pair of PCR primers and a fourth pair of PCR primers, a first member of said third pair of PCR primers hybridizing to [opposite strands] one strand of said nucleic acid sequence and the other member of said third pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said third pair hybridizes [bordering the position of said polymorphic sequence], and a first member of said fourth pair of PCR primers hybridizing to [opposite strands] one strand of said nucleic acid sequence and the other member of said fourth pair of PCR primers hybridizing to the other strand of said nucleic acid sequence on the opposite side of said polymorphic sequence from the side to which said first member of said fourth pair hybridizes [bordering the position of said polymorphic sequence], wherein said PCR primers are [being] characterized as follows:

(i) one of said third pair of PCR primers is (a) [being] complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) [being] non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) [being] non-complementary to said nucleic acid sequence at a single nucleotide that is disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said third pair of primers is capable of amplifying said second allele under appropriate conditions [in its 3'-terminal nucleotides 2-6]; and

(ii) one of said fourth pair of PCR primers is (a) [being] complementary at its 3'-terminal nucleotide to said second allele of said polymorphic sequence, (b) [being] non-complementary at its 3'-terminal nucleotide to said first allele of said polymorphic sequence, and (c) [being] non-complementary to said nucleic acid sequence at one or more nucleotides that is disposed within the five nucleotides adjacent to said 3'-terminal nucleotide, wherein said fourth pair of primers is capable of amplifying said second allele under appropriate conditions [in its 3'-terminal nucleotides 2-6].

17. (Amended) The kit of claim 15, wherein said one of said first pair of PCR primers in step (a)(i) and said one of said second pair of PCR primers in step (a)(ii) [primers of said first and said second primer pairs that comprises a non-complementary nucleotide in its 3'-terminal nucleotides 2-6] also comprise[s] a universal primer binding sequence.

18. (Amended) The kit of claim 15, wherein said one of said first pair of PCR primers in step (a)(i) and said one of said second pair of PCR primers in step (a)(ii) [primers of said first and said second primer pairs that comprises a non-complementary nucleotide in its 3'-terminal nucleotides 2-6] also comprise[s] a unique hybridization tag.